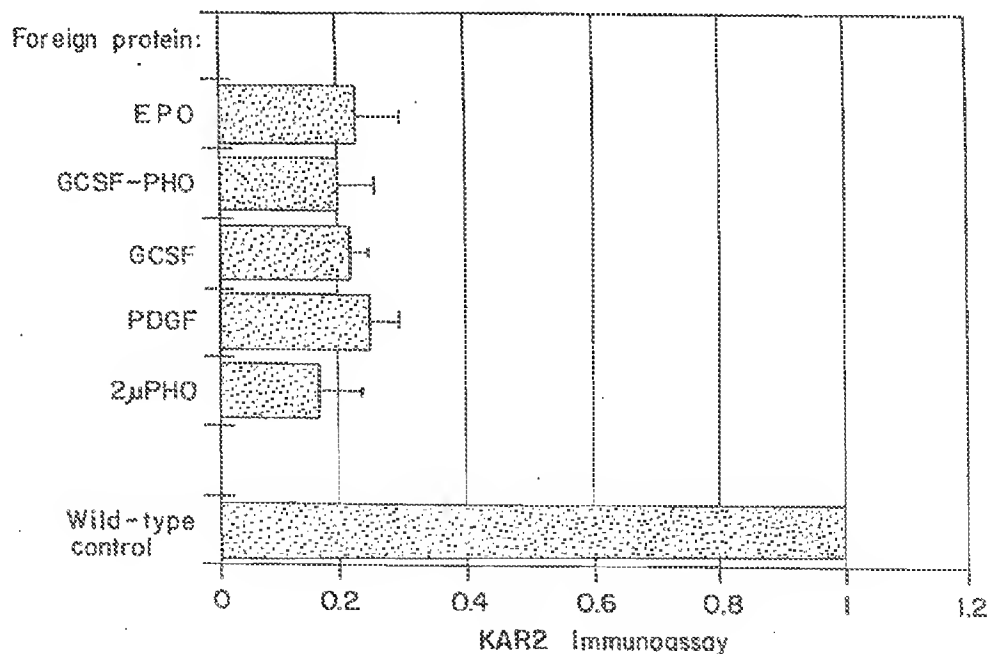




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(54) Title: METHODS FOR INCREASING SECRETION OF OVEREXPRESSED PROTEINS



(57) Abstract

The present invention is directed to methods for increasing secretion of an overexpressed gene product present in a host cell, by inducing expression of chaperone proteins within the host cell.

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EXAMPLE 3INCREASED SECRETION OF OVEREXPRESSED PROTEINS
UPON EXPRESSION OF A CHAPERONE PROTEIN

The KAR2 yeast chaperone coding region was placed under the control of a galactose inducible promoter and the plasmid encoding this chimeric gene was transformed into BJ5464 yeast cells which also carried a plasmid encoding erythropoietin (EPO) under a galactose inducible promoter. These BJ5464 cells were then grown overnight in protein-free glucose medium in the absence of galactose. Expression of KAR2 and EPO proteins was induced by transfer of the BJ5464 cells into a galactose medium (SC GAL).

Cell growth after induction was monitored by observing the optical absorption of the culture at 600 nm. Cell and supernatant samples were taken at 24, 48 and 72 hours after induction. Cell samples were used for determination of KAR2 protein levels using the slot blot procedure described in Example 1. Supernatant samples were tested for the amount of secreted EPO by using the slot blot procedure with a SY14 monoclonal antibody which is specific for EPO.

Fig. 3 depicts the KAR2 expression observed in cell extracts collected at 24, 48 and 72 hours after induction. The KAR2 immunoassay values provided in Fig. 3 represent a ratio of the amount of KAR2 detected in a given yeast cell type relative to wild type yeast. KAR2 expression in wild type cells (*), cells transformed with the EPO-encoding plasmid only (*, GalEpo) and cells transformed with both the EPO-encoding plasmid and the KAR2-encoding plasmid (Δ, GalEpo+GalKar2), is depicted. After induction, expression of KAR2 is initially higher in cells with the EPO-encoding plasmid than in wild type

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1 yeast cells. However, GalEpo cellular expression of
KAR2 drops to almost wild type levels by 48 hours after
induction. If KAR2 expression were monitored for longer
periods of time, the amount of KAR2 in the GalEPO cells
5 would be less than wild type, as shown in Fig. 1.
However, KAR2 expression at 24 hr is significantly
greater in GalEpo+GalKAR2 cells which have the KAR2-
encoding plasmid despite the presence of overexpressed
EPO. Moreover, by 48 to 72 hours after induction, KAR2
10 expression is at least 4- to 5-fold higher in cells
expressing additional amounts of KAR2 recombinantly than
in cells expressing KAR2 from a native, genomic locus.
Therefore, KAR2 expression can be boosted significantly
by recombinant expression.

15 Fig. 4 depicts the growth of wild type cells
(□), cells transformed with the EPO-encoding plasmid
only (O, GalEpo) and cells transformed with both the
EPO-encoding plasmid and the KAR2-encoding plasmid (Δ,
GalEpo+GalKar2) after induction of EPO and KAR2
20 expression.

The inset provided in Fig. 4 depicts the
amount of EPO secreted into the medium of cells which
have the EPO-encoding plasmid only (GalEpo) compared
with the amount of secreted EPO from cells having both
25 the EPO-encoding plasmid and the KAR2-encoding plasmid
(GalEpo+GalKar2). The supernatants tested were
collected during exponential growth of these yeast
strains at the indicated time point (arrow). As shown
in the Fig. 4 inset, the amount of EPO secreted upon
30 induction of KAR2 expression is almost five-fold higher
than when no additional KAR2 chaperone protein is
present.

Therefore, increasing KAR2 expression causes a
substantial increase in protein secretion.

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EXAMPLE 4CONSTRUCTION OF STRAINS OVEREXPRESSING BiP AND PDI

Yeast strains were constructed which overexpress yeast BiP, PDI or both BiP and PDI.

The overexpression system for BiP utilizes the glyceraldehyde-3-phosphate dehydrogenase (GPD) constitutive promoter. A Sall-AatII fragment containing the GPD promoter was ligated into the AatII-Sall site of the pMRI341 expression vector described in Example 2, replacing the galactose (GAL) promoter used for inducible expression of yeast BiP. A single-copy centromere plasmid containing this construct was named pGPDKAR2. BJ5464 cells were transformed with pGPDKAR2.

To construct a yeast strain that overexpresses yeast PDI, an expression cassette containing the yeast PDI gene downstream of the constitutive ADHI promoter was integrated into the chromosomal copy of PDI using LEU2 as a selective marker. Yeast strain BJ5464 with this integrated PDI expression cassette was renamed YVH10 (PDI::ADHI-PDI-Leu2 ura3-52 trp 1 leu2Δ1 his 3Δ200 pep4::H153 prb 1Δ1.6p can 1 GAL).

YVH10 cells were transformed with pGPDKAR2 to provide cells overexpressing both BiP and PDI.

Cells extracts from mid-exponential phase cultures of BJ5464, BJ5464 transformed with pGPDKAR2, YVH10, and YVH10 transformed with pGPDKAR2 were prepared. Yeast BiP and PDI were detected by chemiluminescence using α-Kar2lgG and α-PDIlgG, respectively. Densitometry was performed with an Apple Optical Scanner and analyzed with the program Image (NIH). Quantitation of band intensity was determined from three dilutions of protein and multiple time

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1 exposures of the bands within the linear range of the
film.

As demonstrated in Table 3, BiP was
overexpressed approximately 5-6 fold, and PDI was
5 overexpressed approximately 11-16 fold.

TABLE 3

	BJ5464	BJ5464 +pGPDHAR2	YVH10	YVH10 +GPDHAR2
BiP overexpressed	-	+	-	+
10 PDI overexpressed	-	-	+	+
Densitometry scan, α BiP	1	5.9	1.3	5.5
Densitometry scan, α PDI	1.3	1	16	11

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EXAMPLE 5INCREASED SECRETION OF OVEREXPRESSED PROTEINS
UPON EXPRESSION OF A CHAPERONE PROTEIN

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5 The four yeast strains described in Example 4
(BJ5464, BJ5464 + pGPDKAR2, YVH10, and YVH10 + pGPDKAR2)
are grown for several generations in synthetic complete
(S.C.) media to provide strains which overexpress
neither BiP nor PDI, BiP alone, PDI alone, or both BiP
10 and PDI, respectively. The strains are each transformed
with an expression vector which directs the constitutive
expression of a gene product. Supernatant samples are
collected during exponential growth of the transformed
cells and assayed for the presence of the secreted gene
15 product.

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1 WHAT IS CLAIMED:

1. A method for increasing secretion of an overexpressed gene product from a host cell which comprises effecting the expression of at least one chaperone protein capable of increasing secretion of said overexpressed gene product in said host cell.

2. The method of Claim 1 wherein said expression of said chaperone protein is effected by inducing expression of a nucleic acid encoding said chaperone protein.

3. The method of Claim 2 wherein said nucleic acid is present in an expression vector.

4. A method for increasing secretion of an overexpressed gene product from a host cell which comprises a) effecting the expression of at least one chaperone protein and the overexpression of a gene product in a host cell; and

b) cultivating said host cell under conditions suitable for secretion of said overexpressed gene product.

5. The method of Claim 4 wherein said expression of said chaperone protein is effected by transforming said host cell with an expression vector comprising a nucleic acid encoding said chaperone protein.

6. The method of Claim 5 wherein said overexpression of said gene product is effected by transforming said host cell with an expression vector comprising a nucleic acid encoding said gene product.

7. The method of any one of Claims 1-6 wherein said chaperone protein is an hsp70 chaperone protein or a protein disulfide isomerase.

8. The method of Claim 7 wherein said hsp70 chaperone protein is a KAR2 or a BiP chaperone protein.

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1 9. The method of Claim 7 wherein said protein
disulfide isomerase is a mammalian protein disulfide
isomerase or a yeast protein disulfide isomerase.

5 10. A method for increasing secretion of an
overexpressed gene product from a host cell which
comprises effecting the expression of an hsp70 chaperone
protein and a protein disulfide isomerase protein in
said host cell.

10 11. The method of Claim 10 wherein said host
cell is a yeast cell.

12. The method of Claim 11 wherein said hsp70
chaperone protein is KAR2 and said protein disulfide
isomerase is yeast protein disulfide isomerase.

15 13. A method for increasing secretion of an
overexpressed gene product which comprises transforming
a host cell with an expression vector comprising a
nucleic acid encoding said gene product under conditions
suitable for expression of said gene product, wherein
said host cell is overexpressing at least one chaperone
protein.

20 14. The method of Claim 13 wherein said host
cell is overexpressing an hsp70 chaperone protein and a
protein disulfide isomerase.

25 15. The method of Claim 13 wherein said
chaperone protein is an hsp70 chaperone protein or a
protein disulfide isomerase.

30 16. The method of Claims 14 or 15 wherein
said hsp chaperone protein is KAR2 and said protein
disulfide isomerase is yeast protein disulfide
isomerase.

17. The method of Claim 16 wherein said host
cell is a yeast cell.

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